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Animal Health and Welfare

National Research Council (US) Committee on Defining Science-Based Concerns Associated with Products of Animal Biotechnology, Washington DC, U.S.A.

Introduction

The effects of genetic engineering on animal health and welfare are of significant public concern (Mench, 1999). Ideas about animal welfare are shaped by cultural attitudes toward animals (Burghart and Herzog, 1989), and animal welfare has proven difficult to assess because it is so multifaceted and involves ethical judgments (Mason and Mendl, 1993; Fraser, 1999). The committee considered the following animal welfare aspects of transgenic and cloning technologies: their potential to cause pain, distress (both physical and psychologic), behavioral abnormality, physiologic abnormality, and/or health problems; and, conversely, their potential to alleviate or to reduce these problems. Both the effects of the technologies themselves and their likely ramifications were addressed.

Reproductive Technologies

Reproductive manipulations, including superovulation, semen collection, artificial insemination (AI), embryo collection, and embryo transfer (ET), are used in the production of both transgenic animals and animals produced by nuclear transfer (NT). Commercial livestock breeders also use many of these manipulations routinely. However, while these procedures do raise animal welfare concerns (Matthews, 1992; Moore and Mepham, 1995; Seamark, 1993), these generally are not specific to the production of genetically engineered animals. Few of these procedures have received systematic study from the perspective of animal welfare (Van der Lende *et al*., 2000).
Handling and restraint can be distressful to farm animals (Grandin, 1993) but are essential for almost all husbandry procedures, including those involving reproductive manipulation. Certain reproductive manipulations (e.g., the administration of injections to induce ovulation) can cause additional transient distress, as can electroejaculation.

AI and embryo collection and transfer present a range of animal welfare issues depending on the species used. In cattle, these procedures can be accomplished with minimally invasive non-surgical procedures—the latter under epidural anesthesia. However, in sheep, goats, and pigs these manipulations involve surgical or invasive procedures (laparotomy or laparoscopy), and hence the potential for operative and postoperative pain. In poultry species the hen is killed in order to obtain early-stage embryos. In fish, eggs and milt might be hand-stripped in some species (causing handling discomfort), while in others the males or females must be killed to obtain eggs and/or sperm.

Since breeding livestock are valuable, they might be subjected to these reproductive manipulations repeatedly during their lifetime. In particular, because of the problems involved in screening microinjected embryos prior to implantation to ensure that they actually are carrying the transgene of interest (Eyestone, 1994), recipient cows might be subject to transvaginal amniocentesis for genotyping; nontransgenic fetuses (or male fetuses) are then aborted and the cows reused as recipients (Brink *et al*., 2000). While this limits the number of recipient animals used, it also raises welfare concerns over the repeated exposure of individual animals to procedures likely to cause pain and distress.

Replacements for, or alternatives to, some reproductive manipulations are available (Moore and Mepham, 1995; Seamark, 1993). For example, a method has been devised for non-surgical embryo transfer in pigs, and ova for some purposes can be obtained from slaughterhouses, which eliminates the need for manipulation of live donor livestock females. The use of nuclear transfer to produce transgenic animals could eliminate the problem of repeated elective abortion and reuse of recipient animals, since cell populations with specific genotypes or phenotypes could be selected before embryo reconstruction (Eyestone and Campbell, 1999).

In Vitro Culture

The development of *in vitro* embryo culture techniques has provided an alternative to *in vivo* culture, but ruminants produced by *in vitro* culture methods, whether or not they are carrying a transgene, tend to have higher birth weights and longer gestations than calves or lambs produced by AI (Walker *et al*., 1996; Young *et al*., 1998)—a phenomenon referred to as large-offspring syndrome (LOS). Kruip and den Dass (1997) surveyed researchers worldwide who use *in vitro* reproductive technologies with different breeds of cattle, and also obtained data from a controlled study of Holstein–Friesian calves. The data showed that only 7.4 to 10 percent of calves produced by AI or ET weighed more than 50 kilograms (kg) and only 0.3 to 4.1 percent weighed more than 60 kg, while 31.7 percent of calves produced by *in vitro* procedures (IVP) weighed more than 50 kg and 14.4 percent weighed more than 60 kg. LOS animals have more congenital malformations and higher perinatal mortality rates, although the incidence and severity of the effects reported vary widely among studies (Van Reenen *et al*., 2001). The range of abnormalities reported includes skeletal malformations (Walker *et al*., 1996), incomplete development of the vascular system and urogenital tract (Campbell *et al*., 1996), immune system dysfunction (Renard *et al*., 1999), and brain lesions (Schmidt *et al*., 1996). Even when IVP calves are not excessively large, however, they seem to be less viable and more often experience problems like double-muscling, leg and joint problems, hydroallantois, heart failure, enlarged organs, and cerebellar dysplasia (Mayne and McEvoy, 1993; Schmidt *et al*., 1996; Kruip and den Dass, 1997). In a large-scale study, van Wagtendonk-de Leeuw *et al*. (1998) found that 3.2 percent of calves born after IVP showed congenital abnormalities as compared to only 0.7 percent of calves produced by AI. Hydroallantois and abnormal limbs and spinal cords were especially prevalent.

The mechanism(s) responsible for these effects are unknown, but chromosomal abnormalities and disturbances in the regulation of early gene expression and in communication between the fetus and the recipient mother have been implicated (Barnes, 1999; Van Reenen *et al*., 2001). Cows carrying fetuses produced by IVP show abnormal placental development (Bertolini, 2002). Culture conditions are associated with LOS and other developmental abnormalities, and changing culture conditions (e.g., by not using fetal calf serum and not co-culturing with somatic cells) can help to decrease the rates of LOS and perinatal mortality (Sinclair *et al*., 1999; Van Wagtendonk-de Leeuw

et al., 1998). Oocyte quality also might play a role in LOS and other developmental abnormalities (Kruip *et al*., 2000).

Because of LOS, difficult calvings (dystocia) can be a problem. The mean rate of dystocia across the five breeds represented in the Kruip and den Dass (1997) dataset was 25.2 percent for IVP-produced animals. In the population of Holsteins studied by Kruip and den Dass, dystocia scores were higher (3.05) in IVP than in AI (2.44) or embryo transfer (ET; 2.74) calves, indicating a more difficult delivery in cows carrying IVP fetuses; 14.4 percent of IVP-produced calves died perinatally as compared to 6.6 percent of ET or 6.1 percent of AI calves, and 13 percent of IVP calves were delivered by emergency Cesarean section, as opposed to 0.9 percent of calves produced by standard AI techniques. Because of this, it is becoming more common to deliver IVP offspring by elective caesarian section (Eyestone, 1999). Again, the number of times that this procedure should be performed on any individual animal during her lifetime is an issue of concern. The selection of older, higher parity cows as recipients is important to decrease the incidence of dystocia.

There also is a potential for IVF to have longer-term effects, although detailed data for livestock are lacking (Van Reenen *et al*., 2001). Even though they are heavier at birth, and might have enlarged organs, IVP-produced bulls seem to have normal semen quality and heifers show normal reproductive maturation (Van Wagtendonk-de Leeuw *et al*., 2000). IVP calves have normal growth rates and slaughter weights (Farin and Farin, 1995; McEvoy *et al*., 1998). Studies with mice, however, have shown that *in vitro* manipulation can result in long-term phenotypic changes (Reik *et al*., 1993), including retarded growth and abnormal DNA methylation patterns; these changes can be transmitted to the offspring (Römer *et al*., 1997). Intracytoplasmic sperm injection (ICSI) is under development for fertilizing livestock embryos, and ICSI procedures have been combined with microinjection to produce transgenic animals (Perry *et al*., 1999). A concern is that, since the normal fertilization method of sperm and egg membrane fusion is bypassed—as is the sperm selection that normally would take place in the female reproductive tract (Galli and Lazarri, 1996)—embryos can be produced from abnormal sperm (Liu *et al*., 1995), possibly resulting in abnormal offspring.

Efficiency of Production and Number of Animals Needed

Microinjection is an extremely inefficient method for producing transgenic offspring. Although the success of the method varies by species and gene construct, it has been estimated that less than one percent of microinjected livestock embryos result in transgenic offspring, and, of those, typically fewer than half actually express the transgene (Pursel *et al*., 1989; Rexroad, 1994). Ebert and Schindler (1993) reported efficiencies of between 0 to 4 percent for production of transgenic pigs, cattle, sheep, and goats. About 80 to 90 percent of the mortality occurs very early during development, before the eggs are even mature enough to be transferred to the recipient female (Eyestone, 1994), but postnatal mortality also occurs (Pursel *et al*., 1989).

Even if an individual does express the transgene, it might not be transmitted to subsequent generations. Approximately 30 percent of transgenic mice are mosaics, which means that they carry the transgene in only some of their cells (Wilkie *et al*., 1986). High rates of mosaicism are observed in other animals as well (e.g., fish, Hallerman *et al*., 1990; Gross *et al*., 1992). In one study involving transgenic cattle, seven out of eight transgenic founder males produced by pronuclear DNA injection were mosaics (Eyestone, 1999). Mosaic founder animals might not pass the transgene to their offspring at all, or they might transmit it at a normal or reduced rate.

In mice and pigs, the inefficiency associated with microinjection can be compensated for to a great extent by implanting recipient females with multiple embryos. In cattle, however, this can result in difficult births as well as masculinization of the female offspring (freemartinism) if both a male and a female embryo are transferred. For this reason, embryos usually are cultured temporarily *in vitro* or in recipient cow, sheep, or rabbit oviducts until the stage at which longer-term viability can be established (Eyestone, 1994). If cows are used, these developed embryos need to be recovered and then transferred to the recipient animals. Although this technique requires the use of additional animals for the "culturing" stage, it can reduce the number of recipient cows needed by up to 90 percent.

Mutations

Because inserted DNA can insert itself into the middle of a functional gene, insertional mutations that alter or prevent the expression of that functional gene inadvertently might be generated. Meisler (1992) estimates that 5 to 10 percent of established transgenic mice lines produced by microinjection have such mutations, and it is likely that similar rates would be found in microinjected livestock. Most (about 75 percent) of these are lethal prenatally, but those that are not are responsible for an array of defects in mice, including severe muscle weakness, missing kidneys, seizures, behavioral changes, sterility, disruptions of brain structure, neuronal degeneration, inner ear deformities, and limb deformities. Individuals with such mutations can vary enormously with respect to the degree and type of impairment shown. And because many insertional mutations are recessive, their effects do not become obvious until the animals are bred to transgenic relatives. For example, although mice engineered with a transgene for herpesvirus thymidine kinase were normal, their offspring that were homozygous for the transgene had truncated hind limbs, forelimbs lacking anterior structures and digits, brain defects, congenital facial malformations in the form of clefts, and a greatly shortened life expectancy (McNeish *et al*., 1988).

Many of the problems associated with random-site integration, including insertional mutagenesis, could be circumvented by gene targeting, which allows for the controlled integration of transgenes into predetermined loci within the genome. In addition to site-specific transgene insertions, gene targeting also permits the removal (knockout) and replacement of existing genes. However, problems with the expression of inserted genes still can arise, while the phenotypic consequences of knocking out a gene will depend upon the function of that gene.

Gene Expression

Animal welfare problems also can arise because of poorly controlled expression of the introduced gene. Many transgenic animals either do not express the inserted gene, or show variable or uncontrolled expression (Seamark, 1993; Eyestone, 1999; Niemann *et al*., 1999), although the percentage of inappropriate expression might be decreasing as transgenic technologies are refined. It must be noted that earlier experiments with transgenic growth hormone in pigs used metalothionine promoters. Current approaches use more appropriate promoters with greatly reduced abnormalities, although with methods of pronuclear injection, there are still problems and variability.

The most frequently cited example of welfare problems arising from inappropriate transgene expression is that of the so-called Beltsville pigs, which were engineered with a gene for human growth hormone in an attempt to improve growth rate and decrease carcass fat content (Pursel *et al*., 1987). Backfat thickness was reduced and feed efficiency was improved, although growth rate

was not increased. However, the pigs were plagued by a variety of physical problems, including diarrhea, mammary development in males, lethargy, arthritis, lameness, skin and eye problems, loss of libido, and disruption of estrous cycles. Of the 19 pigs expressing the transgene, 17 died within the first year. Two were stillborn and four died as neonates, while the remainder died between two and twelve months of age. The main causes of death were pneumonia, pericarditis, and peptic ulcers. Several pigs died during or immediately after confinement in a restraint device (a metabolism stall), demonstrating an increased susceptibility to stress. Similar problems are seen in mice transgenic for human growth hormone (Berlanga *et al*., 1993).

Problems due to growth hormone expression also can be seen when the inserted gene comes from the same, or a closely related, species. For example, sheep in which ovine growth hormone inappropriately is expressed are lean but diabetic (Nancarrow *et al*., 1991; Rexroad, 1994). In salmonids transgenic for fish growth hormone (Devlin *et al*., 1995a), the largest transgenic fish have growth abnormalities of the head and jaw. Fish with the highest early growth performance are affected the most and have difficulty eating. As a result, growth of these fish is retarded relative to other transgenics at 15 months of age, and they die prior to maturation. Thus, the severity of morphologic abnormalities is correlated with initial growth rate, although not all transgenic fish display abnormalities. Devlin *et al*. (1995b) also observed that transgenic coho salmon exhibit cranial deformities and opercular overgrowth. After one year of development, the overgrowth of cartilage in the cranial and opercular regions of the fish with this atypical phenotype becomes progressively more severe and reduces viability. Further, all F_1 progeny were deformed seriously, with excessive cartilage growth in the cranium, operculum, and lower jaw, and they had low viability. The deformities in the offspring were more severe than those observed in their parents at the same age. Devlin attributed this to the mosaicism between founder and F_1 generation, with elevated levels produced in the F_1. Devlin *et al*. (1995a) concluded that the best optimal long-term stimulation is achieved in transgenic individuals that show intermediate levels of initial growth enhancement.

As in mice, the genetic background of particular selected strains of farm animals probably is important in determining the severity of the defects associated with the transgene. Pursel *et al*. (1989) speculated that the deformities found in the Beltsville pigs would have been less severe if the foundation stock had been selected for leg soundness and adaptation to commercial rearing conditions.

Uniqueness of Transgenic Animals

Because there can be so much variation in the sites of gene insertion, the numbers of gene copies transferred, and the level of gene expression, every transgenic animal produced by microinjection is (theoretically, at least) unique in terms of its phenotype. Pigs transgenic for growth hormone, for example, vary enormously in the number of DNA copies that they have per cell (from 1 to 490) and in the amount of growth hormone that they secrete (from 3 to 949 nanograms per milliliter, or ng/ml). Half of pigs transgenic for a gene (*c-ski*) intended to enhance muscle development experienced muscle weakness in their front legs, and in general the degree and site of muscle abnormality in these pigs varied considerably from one individual to another (Pursel *et al*., 1992).

This variability makes the task of evaluating the welfare of transgenic animals particularly difficult, since adverse effects almost are impossible to predict in advance, and each individual animal must be assessed for such effects. Van Reenen and Blokhuis (1993) describe the difficulties involved in such assessments. In most cases, deleterious phenotypic changes in transgenic farm

animals—particularly animals transgenic for growth hormone or other growth promoting factors—have been easy to detect because they cause such gross pathologies. However, more subtle effects also are possible. Growth hormone, for example, has many systemic effects, including effects on the efficiency of nutrient absorption, fecundity, and sexual maturation (Bird *et al*., 1994). Growth hormone constructs in salmonids have been shown to influence smoltification (Saunders *et al*., 1998), gill irrigation, disease resistance, body morphometry (Devlin *et al*., 1995a,b), pituitary gland structure (Mori and Devlin, 1999), life span (Devlin *et al*., 1995a,b), and larval developmental rate (Devlin *et al*., 1995b).

Gene insertion and removal also can have effects on behavior—sometimes subtle. For example, growth hormone constructs in fish have been found to affect swimming ability (Farrell *et al*., 1997), feeding rates (Abrahams and Sutterlin, 1999; Devlin *et al*., 1999), and risk-avoidance behavior (Abrahams and Sutterlin, 1999). Some types of knockout mice also have been found to exhibit behavioral problems, such as increased aggressiveness and impaired maternal and spatial behaviors (Nelson, 1997) that are not immediately apparent, but that significantly could affect housing and care requirements.

Sometimes adverse effects are seen only when animals are challenged in some way. The abnormal stress response of the Beltsville pigs, when restrained, is an obvious example. In addition, some problems might not become evident until later in development. Mice transgenic for an immune system regulatory factor, interleukin 4, develop osteoporosis, but not until about two months of age (Lewis *et al*., 1993). This emphasizes the importance of monitoring the welfare of founder transgenic animals, and sometimes successive generations, throughout their lifetime using multiple criteria, including behavioral abnormality, health, and physiologic normality (Van Reenen *et al*., 2001). There has been only a limited number of studies of the welfare of transgenic farm animals to date, and detailed behavioral studies are particularly lacking.

Nuclear Transfer

Somatic cell nuclear transfer (NT) is a relatively new process, and currently is very inefficient. High prenatal mortality and developmental abnormality, LOS, perinatal mortality, and abnormal placentation commonly are reported in cloned cattle and sheep (e.g., Wilson *et al*., 1995; Garry *et al*., 1996; Wells *et al*., 1997; Kato *et al*., 1998; Hill *et al*., 1999; 2000; De Sousa *et al*., 2001). Most mortality in cloned offspring appears to occur within the first few days after birth, although later mortality also is seen. Health and welfare problems reported in the immediate postnatal period include respiratory distress, lethargy, lack of a suckling reflex, cardiomyopathy, pulmonary hypertension, hydroallantois, hypoglycemia, hyperinsulinemia, urogenital tract abnormalities, pneumonia, and metabolic problems. However, such problems are not seen universally in cloned animals; many apparently healthy adult cattle, sheep, and goats have been cloned from adult, fetal, and embryonic cells (Lanza *et al*., 2001; Cibelli *et al*., 2002). For example, Wells *et al*. (1999) succeeded in producing 10 healthy calves from 100 transferred NT blastocysts; the calves were not exceptionally large, all had a strong suckling reflex, and only one required veterinary intervention. Lanza *et al*. (2001) report that the 24 dairy cows surviving from an original group of 30 cloned cattle are in normal physical condition for their stage of production, exhibited puberty at the expected age, have high conception rates after artificial insemination, and show no clinical or immunologic abnormalities.

It is difficult to determine which problems are due to cloning (nuclear transfer) per se, to embryo culture or transfer methods, or to some combination of cloning and culture/transfer methods (Wilson *et al*., 1995; Kruip and den Dass, 1997; Van Wagtendonk-de Leeuw *et al*., 1998).

There is considerable variation among studies in rates of early embryonic death, perinatal mortality, LOS, and dystocia (Kruip and den Dass, 1997; Cibelli *et al*., 2002). The incidence of these problems actually is sometimes lower in animals produced by NT than is typical for animals produced by IVP. Varying levels of expertise and proficiency with the relevant techniques certainly could be contributing factors. Because of their economic value, cloned animals would be expected to receive a high level of veterinary oversight and intervention, which could contribute to the higher postnatal survival of cloned animals in some studies. In cases where there are neonatal problems, they might resolve within a few days of birth (Garry *et al*., 1996).

One possible contributing factor to the high prenatal and neonatal mortality seen in cloned animals is improper epigenetic reprogramming (Young and Fairburn, 2000; Rideout *et al*., 2001). Cloned animals have abnormal methylation patterns, although the significance of this for embryo development and survival in livestock is unclear. The longer-term effects of cloning and/or improper epigenetic reprogramming on animal welfare have yet to be thoroughly evaluated; as the number of surviving cloned livestock increases, such assessments will be possible. There still is a need for detailed behavioral studies of cloned livestock, since cloning has been shown to result in the impairment of mice in learning and motor tasks, although this impairment is transient (Tamashiro *et al*., 2000).

Clones produced by fusion of nuclear donor cells with unfertilized eggs are not identical twins, but "genetic chimeras," since almost all cloned livestock studied to date have mtDNA from the recipient egg but not from the donor cell (Evans *et al*., 1999; Takeda *et al*., 1999). Whether or not there are potential adverse effects on health and welfare due to having nuclear DNA from one source and mtDNA from another are unknown, although mitochondria are responsible for important cellular functions and mitchondrial type theoretically could affect relevant production traits as well. Of course, each time normal fertilization occurs, nuclear genes from the sperm are introduced into a different genetic mitochondrial environment than existed in the cells of the male providing the sperm, so the mixing of nuclear and mitochondrial genes is ubiquitous in nature.

During normal aging, telomere lengths shorten, and this phenomenon has been associated with cell senescence. Normal reproductive processes restore telomere lengths in newborns, but there has been concern about whether this same restoration would be seen in animals cloned from adult cells, or whether such animals instead will age prematurely and possibly develop health problems usually seen in older animals. While shortened telomere lengths were seen in one sheep ("Dolly") cloned from adult somatic cells (Shiels *et al*., 1999), telomere lengths apparently are normal in cattle cloned from adult cells (Lanza *et al*., 2001; Betts *et al*., 2001).

Biomedical Applications

In contrast to genetic manipulation of farm animals for production traits, transgenic manipulation for the production of human pharmaceuticals or transplant organs generally is not intended to cause changes that have physiologic effects on the animals themselves. Thus, although unexpected and undesirable phenotypic effects still can occur as a result of gene insertion or cloning technology, there generally are fewer potential animal welfare concerns associated with the production of transgenic farm animals for biomedical purposes than for agricultural purposes (Van Reenen and Blokhuis, 1993).

Pharmaceuticals

Although there is a potential for producing pharmaceuticals in the eggs, blood, urine, or sperm of farm animals (Lubon, 1998; Sharma *et al*., 1994), the most common method is to produce

transgenic cattle or goats that express the protein of interest in mammary tissue. The recombinant protein then is secreted in milk when the female lactates. This poses problems mainly when those proteins either are expressed in non-mammary tissues (so-called ectopic expression) or when they "leak" out of the mammary gland into the circulation (e.g., Lubon, 1998; Niemann *et al*., 1999). If the protein is active biologically in the species in which it is produced, it can cause pathologies and other severe systemic effects (e.g., Massoud *et al*., 1996). Rigorous regulation of the expression of the transgene thus is necessary to ensure that the animal welfare consequences of milk-borne pharmaceutical production are minimized, but such regulation currently is difficult to achieve. However, even when a pharmaceutical is confined to the mammary tissue, the expression of particular proteins has been associated with premature lactational shutdown in goats (Ebert and Schindler, 1993) and pigs (Shamay *et al*., 1992). In pigs, there was evidence that the mammary tissue developed abnormally due to premature expression of the transgene, and that the condition of the mammary gland might have caused lactation to be painful. Similar concerns arise in the case of blood-borne proteins and nutraceuticals (see below) if the products are produced at levels higher than the animal's normal physiologic levels.

Xenotransplantation

In an attempt to prevent hyperacute rejection of pig organs by humans, pigs have been made transgenic for the expression of human complement proteins, which are involved in regulation of the immune response (Cozzi and White, 1995; Tu *et al*., 1999; Cozzi *et al*., 1997; Byrne *et al*., 1997; Cowan *et al*., 2000). No phenotypic abnormalities have been reported in pigs as a result of the expression of transgenes for these human proteins, although, since the pigs are produced by microinjection, there are the usual inefficiencies in terms of the number of embryos microinjected relative to the number of transgenic animals born (Tu *et al*., 1999; Niemann and Kues, 2000).

Research is underway to produce pigs that, in addition to carrying complement transgenes, have both copies of the gene encoding the enzyme that produces the antigen associated with rejection knocked out. The animal welfare implications of this genetic manipulation are unknown; however, the knockout, which causes changes in cellular carbohydrate structure, potentially could have deleterious physiologic effects on the animals (Dove, 2000) and also render them susceptible to infection with human viruses.

An important animal welfare concern related to xenotransplantation is the management and housing of pigs intended for use as organ sources. To minimize the potential for transmission of disease to human recipients, only specific pathogen free (SPF) pigs are used. SPF research animals are used in other contexts besides xenotransplantation, but their use raises several animal welfare issues. SPF pigs are born by hysterotomy or hysterectomy, and then are reared in isolators for 14 days before being placed in the source herd or in the xenotransplantation facility. The natural weaning age for pigs is about eight weeks (three to four weeks in commercial practice), and piglets subjected to extremely early weaning like this are known to develop abnormal behaviors (Weary *et al*., 1999). Older pigs intended for testing or organ donation might be housed in social isolation in unusually barren (i.e., easily sanitizable) environments. Pigs are extremely social animals that, when given the opportunity, will spend considerable time each day foraging, and that develop abnormal behaviors in confinement if not given the opportunity to root or build nests. In the United Kingdom, the Home Office Code of Practice (Her Majesty's Government, 2000) for organ-source pigs, while recognizing the importance of maintaining biosecure facilities, nevertheless recommends that such pigs be housed in stable social groups, and provided with environmental enrichment such as straw or other material suitable for manipulation. The Code requires

justification if the animals' behavioral needs are to be compromised for a xenotransplantation protocol. There are no comparable standards for pigs intended for xenotransplantation in the U.S., and the lack of standardization of housing and care among U.S. facilities for these pigs is a source of concern. Although there are many forms of environmental enrichment available that are suitable for laboratory-housed pigs (Mench *et al*., 1998), appropriate methods for organ-source pigs require development and evaluation (Orlans, 2000).

Other Biomedical Applications

Farm animals might be genetically engineered for human biomedical applications other than xenotransplantation or the production of pharmaceuticals. Research is underway, for example, to produce a porcine model of cystic fibrosis, and there already are farm animal models for retinal degeneration (Petters *et al*., 1997) and neurodegenerative disease (Theuring *et al*., 1997). As genetic engineering techniques for farm animals improve— particularly such that single base coding changes that are typical of many human genetic diseases can be introduced, and the production and use of farm animal models becomes more economically feasible—it is likely that more models for disease research and toxicity testing will be developed. Discussion of the potential issues raised by these biomedical uses of farm animals is outside the scope of this report. However, the welfare implications will depend upon specific features of the model under study, including any unalleviated pain and suffering associated with the disease process itself, as well as the need for specialized husbandry and veterinary care requirements (Dennis, 2002).

Farming

If genetic technology becomes more efficient and affordable, the primary farming applications of transgenesis and cloning likely will be to produce animals with increased growth, improved feed conversion, leaner meat, increased muscle mass, improved wool quality, improved disease resistance, and increased reproductive potential. The technology also can be used to produce food of improved nutritional quality (nutraceuticals) or appeal.

The primary difference between traditional breeding and genetic engineering is the speed at which change typically occurs (although naturally occurring mutations and recombination events also can cause rapid and dramatic change), and the single-gene nature of genetically engineered change. Traditional methods of selection are more likely to be subject to the checks and balances imposed by natural selection. Many related and apparently unrelated traits are correlated genetically; thus, selective breeding involves selecting for a whole phenotype rather than a single gene product. Because most production and behavioral traits in livestock are polygenic and our understanding of livestock genomes is poor, few traits can reliably and predictably be engineered or introduced by manipulating only one gene (Moore and Mepham, 1995). For this reason, the production of a line of transgenics will require generations of selective breeding after the introduction of gene constructs into the founder generation to ensure that animals display the desired phenotype with few or no undesirable side effects.

However, it is clear that serious welfare problems also have resulted from traditional breeding techniques. Broiler chickens are a case in point. Breeding for increased growth has led to serious physical disabilities, including skeletal and cardiovascular weakness. A large percentage of broilers have gait abnormalities (Kestin *et al*., 1992), and these might be painful, making it difficult for the birds to walk to feeders and waterers. In addition, broiler hens must be severely feed restricted to prevent obesity, and this feed restriction is associated with extreme hunger and a

variety of behavioral problems, including problems with mating behavior and hyperaggressiveness (Mench, 2002; Kjaer and Mench, in press). Traditional selection of pigs for increased leanness has led to increased excitability during handling (Grandin and Deesing, 1998), and selection for high reproductive rates (either by shortening the interval between births or increasing the number of offspring born) or increased lactation also has led to welfare problems. In their report, *The Use of Genetically Modified Animals*, the Royal Society (2001) concluded: "Although genetic modification is capable of generating welfare problems…no qualitative distinction can be made between genetic modification using modern genetic modification technology and modification produced by artificial selection." Several ethical frameworks for evaluating the animal welfare implications of biotechnologies applied to animals have been proposed in an attempt to resolve this difficulty. For example, Rollin (1995) has proposed the use of the "principle of conservation", which states that transgenic and cloned animals developed for agricultural uses should not be worse off than the founder animals or other livestock of the same species under similar housing and husbandry practices.

Potential Animal Welfare Benefits

Genetic engineering certainly has the potential to improve the welfare of farm animals. Decreasing mortality and morbidity by increasing resistance to diseases or parasites, or decreasing responses to ingestion of toxic plants, are obvious examples of welfare benefits, and an area in which some transgenic research is focused (Müller and Brem, 1994; Dodgson *et al*., 1999). It also has been pointed out that transgenic animals might receive a higher standard of care than nontransgenic animals because of their greater economic value (Morton *et al*., 1993). Cloning could be used as a strategy for breed preservation to maintain genes that are important for adaptation and resistance to disease, but equally could result in a further narrowing of the gene pool, with possibly deleterious effects on animal health.

Improving disease resistance to decrease pain and suffering is an application of transgenic technology that has clear animal welfare benefits. But it should be stressed that animal welfare is multifaceted, and this needs to be taken into account when assessing welfare impacts of the application of any technology—not just biotechnology. Important elements of animal welfare include freedom from disease, pain, or distress; physiologic normality; and the opportunity to perform normal behaviors (Broom, 1993). While reducing disease clearly is beneficial, if this also permits animals to be confined more closely, and thus decreases the opportunity for them to perform their normal behaviors, then the net effect on welfare could be negative.

Genetic engineering also could be used to deal with non-disease related welfare problems. It might be possible, for example, to engineer hens that produce only female offspring (Banner, 1995). This would eliminate the problems associated with surplus male chicks, which are killed at the hatchery. The need for the so-called standard agricultural practices like castration and dehorning also could be reduced or eliminated by genetic engineering. Pigs are castrated to prevent boar taint in the meat, but this trait is strongly linked (genetically) and thus is amenable to genetic manipulation. Similarly, horns on cattle, which are removed because they cause injuries to humans and other cattle, are the result of a single gene that could be knocked out by genetic manipulation without affecting other desirable performance traits; genetically polled (hornless) breeds of cattle already are available, and are produced by selective breeding.

Costs Versus Benefits

In making assessments about the production of genetically engineered animals for farming, costs and benefits need to be weighed carefully. When expression of growth hormone is regulated appropriately in transgenic pigs, for example, the increases shown in growth and feed efficiency are modest, and are similar to the increases that can be attained simply by injecting pigs with porcine growth hormone (Pursel *et al*., 1989; Nottle *et al*., 1999). Pursel *et al*. (1989) suggest that centuries of selection for growth and body composition might limit the ability of the pig to respond to additional growth hormone. Indeed, it is possible that we already have pushed some farm animals to the limits of productivity that are possible by using selective breeding, and that further increases only will exacerbate the welfare problems that have arisen during selection.

The potential for reduction in genetic diversity in agricultural species also is posed by inappropriate application of certain biotechnologies. Transgenesis raises such concerns because each transgene integration event results in a genetically unique potential founder and only one founder normally is used to found a transgenic line. This can result in a profound genetic bottleneck unless genetic variability is restored to a production line by purposeful utilization of a mating strategy involving backcrossing of the transgenic line to a large number of distinct, presumably nontransgenic, mates. The effects of cloning are more difficult to anticipate because competing processes are at issue. On the one hand, cloning by its nature produces identical copies of a particular individual, reducing genetic variability relative to what would have been transmitted via conventional breeding. On the other hand, cloning makes it possible to save and utilize genetic variability that would not otherwise be available. For example, cloning could be employed to utilize the genetic resources from a steer that had proven to be a high performing individual. Cryopreserved cells could be utilized as donor material. Moreover, cloning is a tool that actually can be used to increase/maintain genetic variance in some situations quite independently of exploiting castrates (Seidel, Jr., 2001). The tradeoff between the competing processes of loss and gain of genetic variance would be case-specific, and it is hard to quantify in the absence of simulation modeling with validation from field observations. Whatever the mechanism causing it, loss of genetic diversity could limit the potential for future genetic improvement of breeds by selective breeding or biotechnologic approaches. Furthermore, disease could spread through susceptible populations more rapidly than through more genetically diverse populations.

A particularly serious concern that arises is susceptibility of species with low genetic diversity to infectious disease. Diversity of animal populations— particularly at major histocompatibility (MHC) loci—is a major factor preventing spread of disease (particularly viral disease; Xu *et al*., 1993; Schook *et al*., 1996; Kaufman and Lamont, 1996; Lewin *et al*., 1999). Different MHC types recognize different viral or bacterial epitopes encoded by pathogens for presentation to the immune system. In genetically diverse populations, pathogens can evade the immune response only if they adapt to each individual MHC type following transmission from one individual to another. The requirement for this evolutionary process provides a population of animals with significant protection against the spread of infection. Pathogens can evade host immune response more easily in genetically uniform populations (Yuhki and O'Brien, 1990). The consequences of the failure of immunorecognition are illustrated by the deadly epidemics of diseases—such as measles—spread by initial contact between Europeans and isolated New World populations that lacked adequate MHC diversity. Not only could enhanced susceptibility create significant risk for spread of "new" infectious diseases in "monocultures" of cloned or highly inbred animal populations, it also could

create new reservoirs for the spread of zoonotic infections—like new strains of influenza—to humans.

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